EFFECTS OF PULPWOOD FIBERS ON FATHEAD MINNOWS AND WALLEYE FINGERLINGS

Lloyd L. Smith, Jr., Robert H. Kramer, and J. Cameron MacLeod

Paper-mill effluents discharged into streams may limit the capabilities of these waters to produce fish. Possible deleterious materials are derived from chemical effluents, bark, and wood fibers which escape from different processing areas of the plants. Effects of chemical effluents on fish and aquatic organisms have been described in a number of studies, but investigations of the effect of suspended wood fibers on the various stages of fish from eggs to adults have been limited. The purpose of this study was to determine some effects of four types of wood fibers found in paper-mill effluents on fathead minnows (*Pimephales promelas*) and young-of-the-year walleyes (*Stizostedion vitreum vitreum*).

Cole (1) showed that heavy suspensions of paper fiber had no short-term effect on several species of mature fish. Dymond and Delaporte (2), working in a Canadian stream, found that walleyes, smallmouth bass (*Micropterus dolomieui*), and bullheads (*Ictalurus nebulosus*) were absent in areas heavily polluted with paper-mill wastes including fiber, but attributed this absence to chemical conditions which prevented survival of young fish. Herbert and Richards (3) found that rainbow trout (*Salmo gairdneri*) grew more slowly in wood-fiber suspensions under controlled conditions. Smith and Kramer (4), working in the Rainy River, Minnesota, noted that suspended wood fibers did not affect walleye eggs but that *Sphaerotilus natans* growing on eggs inhibited hatching of fry.

The present investigations were carried on under controlled laboratory conditions to determine the influence of short exposures to fibers on mortality rate and sublethal physiological responses.

Procedure

Fathead minnows for all experiments were delivered to the laboratory at two-week intervals by a commercial bait dealer. Fish were held at 55°F (13°C) and fed dry trout food. They were held for six days before each trial at the temperature to be used in the bioassay. Dissolved oxygen concentrations were maintained near saturation during acclimatization. Walleye fingerlings were obtained from Minnesota Department of Conservation rearing ponds five days before they were used in trials. They were transported in pond water held 8 to 10°F (4.5 to 5.5°C) lower than the water from which they were taken, and oxygen was added to maintain the dissolved oxygen level. On arrival at the laboratory 10-mg/l MS-222 (tricaine methanesulfonate) was added to calm the fish during unloading and handling. Laboratory water was added slowly for 24 hr after arrival.

Lloyd L. Smith, Jr., Robert H. Kramer, and J. Cameron MacLeod are, respectively, Professor, Research Fellow, and Research Assistant in the Department of Entomology, Fisheries, and Wildlife, University of Minnesota, St. Paul 1, Minnesota.

Mosquito larvae (*Aedes aegypti*) were cultured and fed in large numbers to walleyes which were acclimatized 24 to 36 hr at the test temperature before each experiment. They were mildly anesthetized during the transfer to the test containers (10-mg/l MS-222).

Test vessels for experiments at oxygen saturation were standard glass fish-egg hatching jars with a bubbler device to circulate the fiber and maintain oxygen concentrations (Figure 1-a). Each jar contained six l of water and from four to ten fish (Table I). Twenty-seven jars were placed in a water bath for experimental runs. Test vessels for bioassays at lowered dissolved oxygen concentrations were plastic egg-hatching jars containing 5.75 l of water and a standpipe with propellor inside to circulate the fiber suspension (Figure 1-b). Jar covers were constructed so that the mixture of air and nitrogen introduced into the water at low pressure (one to two psi) would maintain an oxygen level of an appropriate partial pressure over the water. Dissolved oxygen in the water reached equilibrium with the oxygen content of the controlled atmosphere.

Dissolved oxygen concentrations of water in test vessels were determined with the azide modification of the Winkler method in the experiments at oxygen saturation and with a Jarrell-

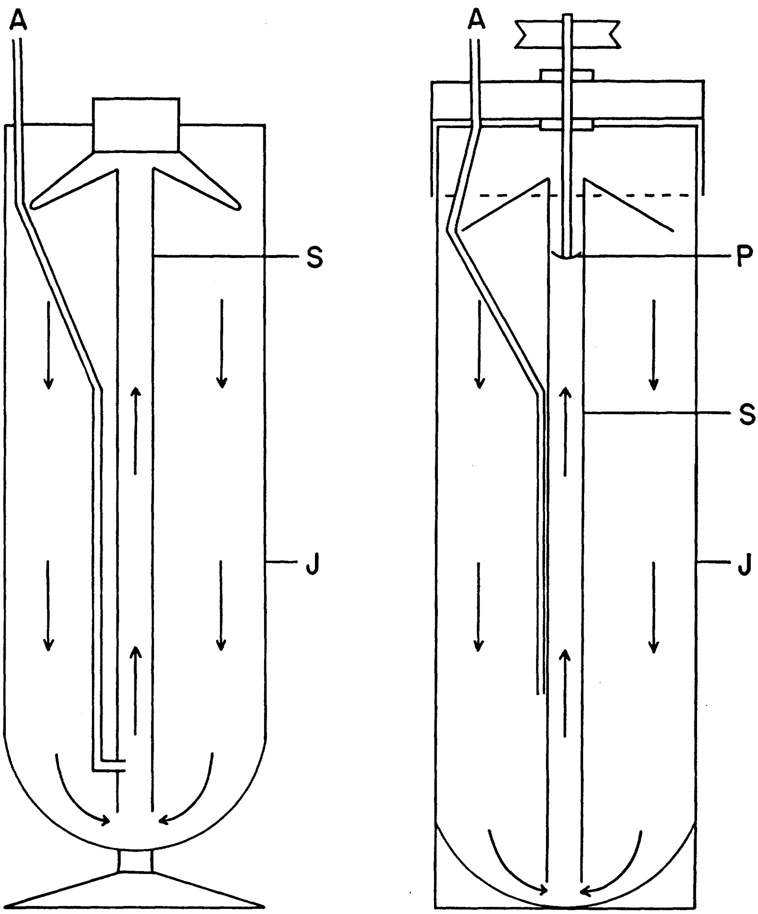

FIGURE 1.—Bioassay vessels. Left, used at oxygen saturation; right, used at reduced oxygen levels. (A) air inlet; (J) cylindrical jar; (P) propellor; (S) standpipe. Arrows indicate water movement.

TABLE I.—Summary of Bioassay Experiments with Fathead Minnows and Walleyes

Bioassay Number	Time (hr)	Species	Total Length (mm)		Temperature (°F)*	Dissolved Oxygen (% saturation)	Number Fish per Jar	Wood Fiber
			Mean	Range				
62-A	72	Walleye	59.0	46–75	65	100	5	Aspen, conifer, sulfite
62-B	72	Walleye	60.8	52–76	75	100	6	Conifer
62-C	72	Walleye	78.9	58–109	75	100	4	Aspen, conifer, sulfite
62-E	72	Walleye	105.9	86–120	65	100	6	Aspen, conifer, sulfite
62-F	72	Walleye	93.2	85–103	75	100	5	Aspen, conifer, sulfite
62-G	96	Fathead minnow	64.2	60–72	65	100	10	Aspen
62-H	96	Fathead minnow	61.3	57–65	75	100	10	Aspen
62-I	96	Fathead minnow	59.6	55–64	55	100	10	Aspen
62-J	96	Fathead minnow	60.3	55–66	55	100	10	Conifer
62-K	96	Fathead minnow	59.5	55–64	65	100	10	Conifer
62-M	96	Fathead minnow	61.9	56–67	55	100	10	Kraft
62-N	96	Fathead minnow	58.1	54–65	65	100	10	Kraft
62-O	96	Fathead minnow	61.9	56–70	75	100	10	Kraft
62-P	96	Fathead minnow	61.2	56–68	75	100	10	Conifer
62-Q	96	Fathead minnow	59.8	55–65	65	100	10	Sulfite
62-S	96	Fathead minnow	60.8	55–66	65	100	10	Aspen, conifer, kraft, sulfite
63-B	72	Walleye and fathead minnow	77.0	67–96	65	65	5	Aspen, conifer, sulfite
63-C	72	Fathead minnow	62.5	58–66	65	40	5	Aspen, conifer, sulfite
63-D	72	Walleye	72.2	62–79	65	35	5	Aspen, conifer, sulfite
63-E	72	Walleye	78.5	69–93	75	40	5	Aspen, conifer, sulfite
63-F	72	Fathead minnow	58.9	55–64	75	35	5	Aspen, conifer, sulfite

* Note: °C = (°F − 32) 0.555.

Ash dissolved oxygen analyzer in the experiments at lowered oxygen.

Hematocrits were determined, using methods described by Snieszko (5), from blood of fish which survived to the end of each bioassay. The caudal peduncle was severed and the blood sample was taken in a microhematocrit tube and centrifuged. Upper lethal temperature tolerances were determined for fish surviving to the end of some experiments. Blood iron, oxygen consumption, carbon dioxide evolution, total serum proteins, and lactic acid were determined, and spectrophotometric analysis of hemoglobin was made on survivors of some bioassays.

Four types of wood fibers were used for bioassays: aspen groundwood, conifer groundwood, conifer kraft, and conifer sulfite. Wood fibers for all experiments were supplied by pulp and paper mills. Conifer groundwood (75 percent spruce, *Picea mariana* and *P. glauca,* 25 percent balsam fir, *Abies balsamea*) was obtained immediately after it left the grinder at the St. Regis Paper Company, Sartell, Minnesota. Other wood fibers were supplied by Minnesota and Ontario Paper Company, International Falls, Minnesota. Aspen groundwood (*Populus tremuloides*) was taken from the headbox of the insulite machines; conifer kraft (*Pinus Banksiana*) from lapped pulp (50-percent dry) and slush to decker; and conifer sulfite (mixture of *Picea mariana* and *P. glauca*) from decker and discharge from Cowan screens. All fibers were washed a minimum of four times in the laboratory, aliquots were dried at 80°C at 20-in. vacuum, and dry weights used in preparation of concentrations prepared for bioassays.

Effect of Fiber on Survival

Bioassays at Oxygen Saturation

Fathead Minnows—Ninety-six-hour bioassays on fathead minnows were run from January 15 to April 6, 1962, with 0, 100, 272, 738, and 2,000 mg/l aspen groundwood, conifer groundwood, conifer sulfite, and conifer kraft fibers at oxygen saturation and 55°, 65°, and 75°F (13°, 18°, and 24°C) (Tables I and II). Fifty or 60 fish were used at each fiber level and temperature. Dead fish were removed at 4, 8, 12, 24, 36, 48, 72, and 96 hr. Highest mortality was within the first 12 hr, and few fish died after 72 hr. Oxygen concentrations during these experiments varied from 85- to 110-percent saturation; total alkalinity from 220 to 325 mg/l; and pH from 8.2 to 8.7. At concentrations of 100 and 272 mg/l of all four fibers and at all temperatures, survival was the same as in controls (Table II). Survival of fish tested in 2,000 mg/l conifer groundwood was 22 to 88 percent; in conifer kraft, 76 to 86 percent; conifer sulfite, 94 percent; and aspen groundwood, 96 to 98 percent. At 738 mg/l of various fibers survival varied appreciably from controls only in conifer groundwood (72 to 100 percent). An

TABLE II.—Survival of Fathead Minnows in Four Wood Fibers at Various Oxygen Levels* and Temperatures—Expressed as Percentage

(Each number represents 50 fish, unless otherwise noted in parentheses)

Bioassay Number	Temperature (° F)	Oxygen (percent saturation)†	Wood-fiber Concentration (mg/l)					
			Control 0	74	100	272	738	2,000
Aspen groundwood								
63-B	65	65	100(5)	100(5)	100(5)	—	—	—
63-C	65	40	90(10)	70(10)	60(10)	—	—	—
63-F	75	35	70(10)	80(10)	90(10)	90(10)	—	—
62-I	55	100	100	—	100	100	98	96
62-G	65	100	98	—	100	100	100	98
62-S	65	100	100	—	—	—	94	—
62-H	75	100	100	—	100	100	100	96
Conifer groundwood								
63-B	65	65	100(5)	100(10)	100(5)	100(5)	—	—
63-C	65	40	90(10)	80(10)	90(5)	80(10)	—	—
63-F	75	35	70(10)	80(10)	80(10)	100(10)	—	—
62-J	55	100	96	—	100	100	100	88
62-K	65	100	100	—	100	98	90	33
62-S	65	100	100	—	—	—	78	—
62-P	75	100	100	—	98	96	72(60)	22(60)
Conifer kraft								
62-M	55	100	100	—	100	100	98	80
62-N	65	100	100	—	100	100	98	86
62-S	65	100	100	—	—	—	96	—
62-O	75	100	92	—	90	90	88(60)	76(60)
Conifer sulfite								
63-B	65	65	100(5)	100(10)	100(5)	—	—	—
63-C	65	40	90(10)	70(10)	90(10)	—	—	—
63-F	75	35	70(10)	70(10)	90(10)	80(10)	—	—
62-Q	65	100	96	—	98	98	98	94
62-S	65	100	100	—	—	—	100	—

* 72-hr bioassays at low oxygen levels; 96-hr bioassays at 100-percent saturation.
† Levels designated as 100 percent varied from 85 to 110 percent during course of the run.
Note: °C = (°F − 32) 0.555.

TABLE III.—Survival of Walleye Fingerlings in Three Wood Fibers at Various Oxygen Levels*—Expressed as Percentage

(Number of fish in parentheses)

Bioassay Number	Temperature (° F)	Oxygen (percent saturation)	Wood-fiber Concentration (mg/l)					
			Control 0	74	100	272	738	2,000
Aspen groundwood								
63-B	65	65	80(5)	40(5)	20(5)	—	—	—
63-D	65	35	100(10)	70(10)	40(10)	—	—	—
63-E	75	40	80(10)	30(10)	10(10)	10(10)	—	—
62-A	65	100	86(14)	—	100(10)	60(10)	60(10)	40(10)
62-E	65	100	78(18)	—	25(12)	—	—	—
62-C	75	100	92(12)	—	90(10)	88(8)	100(8)	—
62-F	75	100	100(15)	—	87(15)	—	—	—
Conifer groundwood								
63-B	65	65	80(5)	—	0(5)	—	—	—
63-D	65	35	100(10)	10(10)	10(10)	0(10)	—	—
63-E	75	40	80(10)	20(10)	0(10)	0(10)	—	—
62-A	65	100	86(14)	—	90(10)	0(10)	0(10)	0(10)
62-E	65	100	78(18)	—	33(18)	0(12)	0(12)	—
62-B	75	100	100(6)	—	100(6)	50(6)	—	—
62-C	75	100	92(12)	—	67(12)	0(8)	0(8)	—
62-F	75	100	100(15)	—	67(15)	—	—	—
Conifer sulfite								
63-B	65	65	80(5)	—	40(5)	60(5)	—	—
63-D	65	35	100(10)	30(10)	80(10)	—	—	—
63-E	75	40	80(10)	80(10)	30(10)	10(10)	—	—
62-A	65	100	86(14)	—	93(15)	60(10)	44(9)	30(10)
62-E	65	100	78(18)	—	44(18)	12(17)	6(18)	0(18)
62-C	75	100	92(12)	—	92(12)	88(8)	75(8)	88(8)
62-F	75	100	100(15)	—	100(15)	—	—	—

* 72-hour bioassays.
Note: °C = (°F − 32) 0.555.

increase in temperature of the bioassay from 55° to 75°F (13° to 24°C) lowered the survival only in conifer groundwood where survival decreased from 88 to 22 percent at 2,000 mg/l and from 100 to 72 percent at 738 mg/l.

Walleye Fingerlings—Seventy-two-hour bioassays were conducted from July 9 through August 9, 1962, at oxygen saturation with aspen groundwood, conifer groundwood, and conifer sulfite. Tests were conducted at oxygen saturation, temperatures of 65° and 75°F (18° and 24°C), and at the same fiber concentrations as those used with fathead minnows. All fibers lowered the survival of fish below that of controls in concentrations of 100 mg/l and greater (Table III). At 65°F (18°C) no fish survived in concentrations of conifer groundwood greater than 100 mg/l; 40 percent survived in 2,000 mg/l aspen groundwood; and an average of 11 percent survived in 2,000 mg/l conifer sulfite. Survival was slightly higher at 75°F (24°C) than at 65°F (18°C) in all fibers.

Bioassays at Reduced Oxygen Concentrations

A short series of 72-hr bioassays was run on fathead minnows and walleye fingerlings from July 23 to August

30, 1963, with dissolved oxygen levels reduced to 35-, 40-, or 65-percent saturation and at 65° and 75°F (18° and 24°C). Aspen groundwood, conifer groundwood, and conifer sulfite fibers were used at concentrations of 74, 100, and 272 mg/l. Survival of fathead minnows in all fiber concentrations and oxygen levels was not significantly different from controls (60 to 100 percent, Table II).

Survival of walleye fingerlings varied from 80 to 100 percent in controls (Table III) and from 0 to 80 percent in the fiber treatments. Lowest survival was in 100- and 272-mg/l conifer groundwood where none survived at 75°F (24°C); at 65°F (18°C) 10 percent survived in 100 mg/l and none at 272 mg/l. In 74 mg/l 10 to 20 percent survived. Survival in 100-mg/l aspen groundwood varied from 10 to 40 percent and in 74 mg/l from 30 to 70 percent. Highest survival was 30 to 80 percent in conifer sulfite at 74 and 100 mg/l and 10 and 60 percent in 272 mg/l.

Effect of Fiber on Hematocrit

Hematocrit was determined in 75-mm microhematocrit tubes commercially prepared with ammonium heparin for fathead minnows, in which clotting was not a problem. With walleyes, however, 70 percent of the blood sample clotted in such capillary tubes. Clotting was prevented by the addition of about 10 mm of sodium heparin (10,000 U.S.P. preparation) into the capillary. The length of the heparin column was then measured, half of the blood sample was taken, and the tube was inverted and filled from the opposite end. The end of the capillary was sealed with modelling clay and centrifuged for 10 min. Fish were anesthetized in 1,000-mg/l MS-222 until it was determined that this treatment resulted in 17 percent less blood taken from each fish and that hematocrits were not different from those in un-anesthetized fish (Table IV). No difference in hematocrits was found between sexes in fathead minnows; walleye fingerlings were not classified by sex.

Linear regressions ($Y = a + bX$) were fitted to data where Y = hematocrit and $X = \log_{10}$ fiber concentration in parts per million. A unit increase in X represents a 0.434 increment in logarithm of treatment level. The slope b, therefore, represents the change in hematocrit with each increase in fiber concentration used, i.e., from 100 to 272 mg/l, 272 to 738 mg/l, and 738 to 2,000 mg/l.

Hematocrits at Oxygen Saturation

Hematocrits of fathead minnows increased as fiber concentrations increased with aspen and conifer groundwoods at 55°, 65°, and 75°F (13°, 18°, and 24°C) and with conifer kraft at 65°F (18°C) (Figure 2, Table V). Slopes of regression lines fitted by least squares were significantly different from zero (1- or 5-percent level)

TABLE IV.—Effect of Tricaine Methanesulfonate (MS-222) on Hematocrit and Amount of Blood Collected from Fathead Minnows

Group	Number of Fish	Hematocrit		Length of Blood Column (mm)	
		Mean	S.D.	Mean	S.D.
Group 1					
Anesthetized	12	49.9	5.7	—	—
Not anesthetized	11	46.3	11.8	—	—
Group 2					
Anesthetized	23	41.7	6.2	35.7	6.2
Not anesthetized	24	44.9	8.0	43.0	4.4

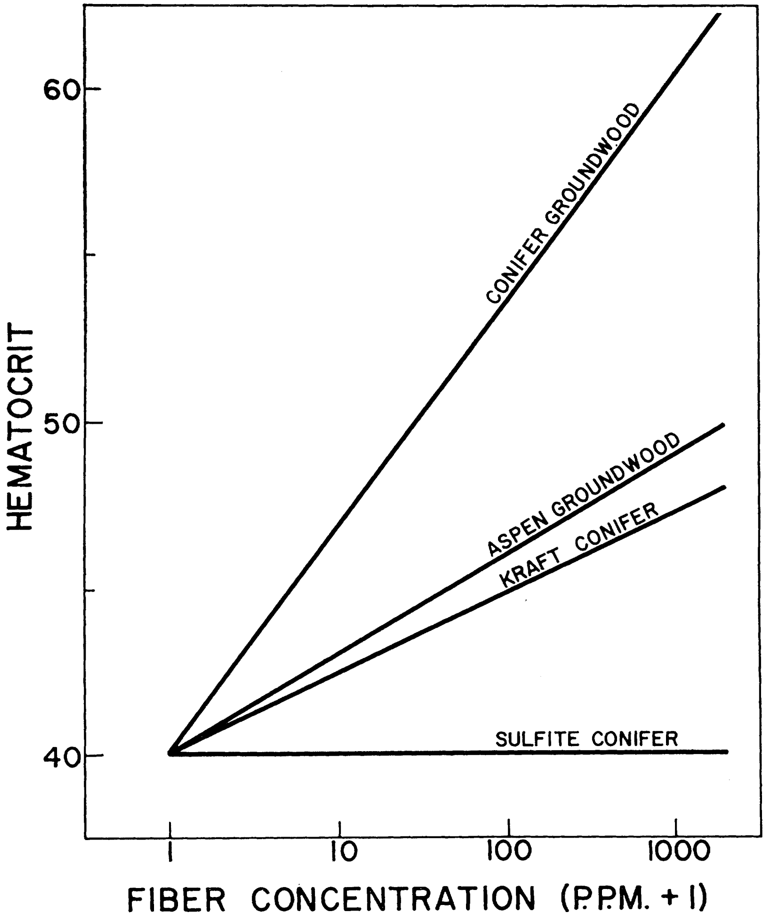

FIGURE 2.—Effect of four wood fibers on hematocrit of fathead minnows, 96 hr, 65°F. Regression lines adjusted to common origin.

and varied from 0.72 for aspen groundwood at 75°F (24°C) to 2.88 for conifer groundwood at 65°F (18°C) (Table V). The slope of the regression for conifer groundwood at 65°F (18°C) (2.88) was significantly different (5-percent level) from the slope at 65°F (18°C) for either aspen groundwood (1.32) or conifer kraft (1.09) and significantly different from the slope for conifer groundwood at 75°F (24°C) (1.54). Results from bioassay 62-S with all four fibers at 738 mg/l and 65°F (18°C) supported these findings in that mean hematocrit was significantly different (5-percent level) from the controls (44) only in the case of conifer groundwood (55). Standard deviation of hematocrits in individual fiber treatments varied from 4.0 to 11.2, but there were no trends in differences among treatments or treatment levels.

Hematocrits of walleye fingerlings increased significantly with fiber concentration only in bioassays with conifer groundwood at 75°F (24°C) and with conifer sulfite at 65°F (18°C). Slopes of regressions in these experiments varied from 0.78 to 1.52 and were not significantly different from each other (Table VI). In bioassay 62-E, hematocrits of walleyes held in 100 mg/l aspen and conifer groundwoods at 65°F (18°C) and in 100 mg/l conifer sulfite at 75°F (24°C)

TABLE V.—Mean Hematocrits of Fathead Minnows Surviving 96-hr Bioassays with Four Fibers at Three Temperatures and Dissolved Oxygen at Saturation

Bioassay Number	Wood Fiber	Temperature (° F)	Wood-fiber Concentration (mg/l)					Slope of Regression
			0	100	272	738	2,000	
62-I	Aspen groundwood	55	43	47	50	53	53	1.36†
62-G	Aspen groundwood	65	38	41	47	45	48	1.32†
62-S	Aspen groundwood	65	44	—	—	46	—	—
62-H	Aspen groundwood	75	42	41	47	48	50	0.72*
62-J	Conifer groundwood	55	43	52	52	56	60	2.01†
62-K	Conifer groundwood	65	41	55	62	58	63	2.88†
62-S	Conifer groundwood	65	44	—	—	55	—	—
62-P	Conifer groundwood	75	38	50	48	45	52	1.54†
62-M	Conifer kraft	55	47	49	53	47	53	—
62-N	Conifer kraft	65	39	42	43	49	46	1.09†
62-S	Conifer kraft	65	44	—	—	47	—	—
62-O	Conifer kraft	75	39	45	44	40	46	—
62-Q	Conifer sulfite	65	41	44	40	36	38	—
62-S	Conifer sulfite	65	44	—	—	44	—	—

* Significant at 5-percent level.
† Significant at 1-percent level.
Note: °C = (°F − 32) 0.555.

(bioassay 62-F) were significantly higher than those of controls (t test, 5-percent level). Standard deviations of hematocrits within individual treatments varied from 2.6 to 8.3, and there were no trends in these differences among wood fibers, concentrations, or temperatures.

TABLE VI.—Mean Hematocrits of Walleye Fingerlings Surviving 72-hr Bioassays with Three Fibers at Two Temperatures and Dissolved Oxygen at Saturation

Bioassay Number	Wood Fiber	Temperature (° F)	Wood-fiber Concentration (mg/l)					Slope of Regression
			0	100	272	738	2,000	
62-A	Aspen groundwood	65	27	31	28	25	—	—
62-E	Aspen groundwood	65	46	51	—	—	—	—
62-C	Aspen groundwood	75	34	40	28	27	—	—
62-F	Aspen groundwood	75	43	38	—	—	—	—
62-A	Conifer groundwood	65	27	29	—	—	—	—
62-E	Conifer groundwood	65	46	51	—	—	—	—
62-B	Conifer groundwood	75	28	37	34	—	—	1.52*
62-C	Conifer groundwood	75	34	33	—	—	—	—
62-F	Conifer groundwood	75	43	37	—	—	—	—
62-A	Conifer sulfite	65	27	30	27	31	39	0.89*
62-E	Conifer sulfite	65	46	51	52	44‡	—	0.78†
62-C	Conifer sulfite	75	34	33	34	28	32	—
62-F	Conifer sulfite	75	43	35	—	—	—	—

* Significant at the 1-percent level.
† Significant at the 5-percent level.
‡ One fish.
Note: °C = (°F − 32) 0.555.

Hematocrits at Reduced Oxygen Levels

Phillips (6) has shown that asphyxia increased red cell counts in trout. In the present study hematocrit increased from 36 to 46 in fathead minnows held for 144 hr at 95-percent and 12-percent oxygen saturation and 60°F (15.5°C) as follows:

Percentage oxygen saturation	Mean hematocrit (n = 18)
12	46
25	41
50	38
95	36

This observed increase in hematocrit due to lowered oxygen levels may have masked fiber effect on fathead minnows since none was found in aspen groundwood, conifer groundwood, and conifer sulfite with concentrations up to 272 mg/l (Table VII).

Hematocrit of walleye fingerlings was significantly increased only in conifer sulfite at 65°F (18°C) (Table VIII). The dissolved oxygen concentration during this bioassay was 65 percent, whereas all other bioassays at low oxygen were run at 35-percent or 40-percent saturation.

Effect of Fiber on Resistance to High Temperatures

The effect of suspended fiber on the resistance of fathead minnows to high temperatures was measured on fish which survived to the end of the 96-hr fiber bioassays. Five to 15 fish taken from a single fiber level were placed in a fiberglass screen container 5 in. (12.7 cm) in diam and 10 in. (25.4 cm) deep. During a single trial fish from the five fiber levels were placed simultaneously in a fiberglass tub containing continuously-aerated water. The water temperature was raised at about 0.75°F (0.4°C) per minute until the lethal temperature was approached, and then the rate was reduced to about 0.15°F (0.08°C) per minute. Water temperatures and time to death for each fish were recorded. A fish was considered dead when it did not respond to prodding with a forceps. Average lethal temperatures for fish subjected to all fibers combined, at 55°, 65°, and 75°F (13°, 18°, and 24°C), and then tested in water without fiber, were 87°, 91°, and 95°F (31°, 33°, and 35°C), respectively.

The data from individual trials were found suited to linear regression analysis. In the equation $Y = a + bX$, Y = the time to death in minutes and X = $\log_{10}$ fiber concentration in parts per million. The slope b represents the decrease in time to death with increase in fiber concentration. Regression

TABLE VII.—Mean Hematocrits of Fathead Minnows Surviving 72-hr Bioassays with Three Fibers at Two Temperatures and at Lowered Oxygen Concentrations

Bioassay Number	Wood Fiber	Temperature (° F)	Oxygen (percent saturation)	Wood-fiber Concentration (mg/l)			
				0	74	100	272
63-B	Aspen groundwood	65	65	48	48	40	—
63-C	Aspen groundwood	65	40	46	50	48	—
63-F	Aspen groundwood	75	35	40	40	39	—
63-B	Conifer groundwood	65	65	48	44	54	47
63-C	Conifer groundwood	65	40	46	48	53	51
63-F	Conifer groundwood	75	35	40	43	44	44
63-B	Conifer sulfite	65	65	48	43	52	—
63-C	Conifer sulfite	65	40	46	52	50	—
63-F	Conifer sulfite	75	35	40	39	35	43

Note: °C = (°F − 32) 0.555.

TABLE VIII.—Mean Hematocrits of Walleye Fingerlings Surviving 72-hr Bioassays with Three Fibers at Two Temperatures and at Lowered Oxygen Concentrations

Bioassay Number	Wood Fiber	Temperature (° F)	Oxygen (percent saturation)	Wood-fiber Concentration (mg/l)			
				0	74	100	272
63-B	Aspen groundwood	65	65	38	44	46†	—
63-D	Aspen groundwood	65	35	35	38	41	—
63-E	Aspen groundwood	75	40	39	42	43†	39†
63-B	Conifer groundwood	65	65	38	—	—	—
63-D	Conifer groundwood	65	35	35	39†	38†	—
63-E	Conifer groundwood	75	40	39	34	—	—
63-B*	Conifer sulfite	65	65	38	—	42	52
63-D	Conifer sulfite	65	35	35	35	34	—
63-E	Conifer sulfite	75	40	39	41	39	40†

* Slope of regression 2.01 (significant at 5-percent level).
† One fish.
Note: °C = (°F − 32) 0.555.

analysis of each trial determined whether the slope *b* was significantly less than zero (Table IX). Conifer sulfite fiber at 65°F (18°C) and conifer kraft at 75°F (24°C) and one trial of conifer groundwood at 75°F (24°C) had no significant effect on high temperature resistance, but all other trials with conifer groundwood and conifer kraft indicated a significant reduction in resistance to high temperatures.

In the experiments in which the effect was significant, the fish treated in 2,000 mg/l fiber died on the average 17 percent sooner than did fish in 0 mg/l, and the upper lethal temperature was on the average 1°F (0.55°C) lower.

Other Studies

Survivors of bioassays with aspen groundwood and conifer kraft fibers were used for analyses of blood lactic acid, blood iron, hemoglobin, electrophoretic patterns of serum proteins, total serum proteins, spectral (absorption) patterns of blood, and respiratory quotients. Methods for determination of lactic acid, iron, and hemoglobin as described by Hawk, Oser, and Summerson (7) were used. Serum protein analyses were made with the paper-strip electrophoresis method described by Patel, Haydak, and Gochnauer (8), and dissolved oxygen and carbon dioxide by procedures outlined in Standard Methods, 11th edition (9). Spectral analyses were made by measuring percentage transmittance of blood in 1-percent saline at 10-μ intervals from 400 to 800 μ.

Significant relationships or trends were not observed. It was noted, however, that within any particular level of fiber treatment individual fish with higher hematocrits invariably had higher serum protein concentrations. Small sample sizes and inherent vari-

TABLE IX.—Significance of Fiber Treatment in Reducing Time to Death at Lethal Temperature

(Regressions based on five fiber levels)

Bioassay Number	Wood Fiber	Bioassay Temperature (° F)	Significance Level of Regression (%)
62-J trial (a)	Conifer groundwood	55	1
62-J trial (b)	Conifer groundwood	55	1
62-K	Conifer groundwood	65	1
62-P trial (a)	Conifer groundwood	75	NS
62-P trial (b)	Conifer groundwood	75	5
62-M	Conifer kraft	55	1
62-O	Conifer kraft	75	NS
62-Q	Conifer sulfite	65	NS

NS Not significant

ability in fish and techniques used may have prevented definitive results.

Conclusions

The effect of suspended wood fibers on mortality of fish depends on the species of fish, type of wood fiber, processing method, dissolved oxygen concentration and, to a lesser degree, water temperature. Conifer groundwood was the most lethal fiber and had the greatest effect upon walleye fingerlings, the less resistant species studied. Groundwood pulps were more lethal than chemical pulps. At lowered oxygen levels, mortality rate of walleye fingerlings was increased in both groundwood and chemical fibers.

Sublethal effects such as change in hematocrit showed that there are significant changes in the blood of fish exposed to wood fibers. Increased hematocrit resulted from increased concentrations of all groundwood fibers when dissolved oxygen was at saturation. At lowered oxygen levels, however, hematocrits were higher due to the reduced oxygen, and any fiber effects were probably masked. Analysis of blood constituents suggested that other qualitative and quantitative changes were present, but the relationship to fiber treatment was not clear. More detailed study of blood chemistry may reveal sublethal effects of fiber exposure which are even more significant than those shown here. Comprehensive evaluation of the influence of sustained fiber exposure at sublethal levels will require study of all life history phases and physiological responses, especially growth, fecundity, and metabolic rates.

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